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# Quality of Vegetable Waste Silages Treated with Various Carbohydrate Sources

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## ABSTRACT

**The aim of this research was to evaluate the quality of vegetable waste silages, using rice bran, *onggok*(cassava flour waste) and pollard as carbohydrate sources. Vegetable waste was collected from local traditional market, consisted of corn husk, chinese cabbage and cabbage. Research was held in randomized block design consisted of six treatments with 3 replications. Treatments were (T1) vegetable waste + rice bran, (T2) vegetable waste + rice bran + rice straw, (T3) vegetable waste + onggok, (T4) vegetable waste + onggok + rice straw, (T5) vegetable waste + pollard, (T6) vegetable waste + pollard + rice straw. *Lactobacillus plantarum*1A-2 was used as innoculant. The quality of silages was evaluated by measuring pH, temperature, population of lactic acid bacteria and lactic acid production. Nutrient characteristic was determined by proximate and fiber analysis. Results showed that pH of silages were not affected by treatments, but silage treated with rice bran, with or without rice straw addition, had higher temperature compared with others (29 $^o$C or 28.3 $^o$C). The highest population of lactic acid bacteria (1.65 x $10^9$ cfu/g) was found in silage using rice straw and *onggok*(T4), but the highest lactic acid production (0.41%) was measured in silage using rice straw and rice bran (T2). In general, the use of rice bran as carbohydrate sources gave the highest lactic acid production followed by pollard and *onggok*. Different carbohydrate source gave different nutrients characteristic. Although the result was not significantly different, silage with highest protein content was measured in silage with pollard as carbohydrate source, followed with rice bran and *onggok*. The result showed that all carbohydrate sources used in this experiment can be used as silage ingredient resulting in good vegetable waste silage.**

***Key words: Lactobacillus plantarum 1A-2, onggok, rice bran, pollard, vegetable waste silages***

## ABSTRAK

**Tujuan dari penelitian ini adalah untuk mengevaluasi kualitas silase limbah sayuran pasar yang menggunakan sumber karbohidrat yang berbeda yaitu dedak padi, onggok dan pollard. Limbah sayuran pasar diperoleh dari pasar tradisional, terdiri atas kulit jagung, sawi putih, dan kol. Penelitian dilakukan menggunakan rancangan acak kelompok (RAK) dengan 6 perlakuan dan 3 ulangan. Perlakuan yang digunakan adalah (P1) limbah sayuran pasar + dedak padi, (P2) limbah sayuran pasar + dedak padi + jerami padi, (P3) limbah sayuran pasar + onggok, (P4) limbah sayuran pasar + onggok + jerami padi, (P5) limbah sayuran pasar + pollard, (P6) limbah sayuran pasar + pollard + jerami padi. Bakteri asam laktat yang digunakan sebagai inokulum adalah *Lactobacillus plantarum* 1A-2. Parameter yang digunakan adalah pH, suhu, populasi bakteri asam laktat dan total asam. Kualitas nutrisi silase diukur menggunakan analisis proksimat dan serat. Hasil menunjukkan bahwa perlakuan yang diberikan tidak mempengaruhi pH, namun silase yang menggunakan dedak padi mempunyai suhu yang paling tinggi. Populasi bakteri asam laktat yang paling tinggi (1,65 x $10^9$ cfu/g) terdapat pada silase yang menggunakan onggok dan jerami padi (P4). Produksi total asam tertinggi diperoleh dari silase yang menggunakan dedak padi dan jerami padi (T2). Secara umum, silase yang menggunakan dedak padi menghasilkan total asam yang paling tinggi. Silase dengan kandungan protein paling tinggi adalah silase yang menggunakan pollard sebagai sumber karbohidrat, meskipun tidak berbeda secara signifikan. Hasil penelitian menunjukkan bahwa semua sumber karbohidrat yang digunakan pada penelitian ini dapat dijadikan bahan campuran silase.**

***Kata kunci: Lactobacillus plantarum 1A-2, onggok, dedak padi, pollard, silase limbah sayuran***

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## INTRODUCTION

Forage is major feed for ruminant but increasingly difficult to obtain due to competing land uses with human interests. Therefore, it is necessary to find new resources that can substitutes the forage. Vegetables waste is a potential feed source for ruminant, to overcome lack of grass especially in dry season. Vegetable waste is part of the vegetables that are not consumed by humans, usually already discarded by traders so has no economic value. Traditional farmers already give vegetable waste to their cattle, but its high water content made it easily decayed and cannot be stored for long period. This limitation increased cost and difficulties in handling because farmers had to take vegetable waste from traditional market everyday. For this reason, it is necessary to preserve vegetable waste and to improve the quality and the nutritional value of vegetable waste as feed.

Ensiling is a method of preserving moist forage preservation that is widely used all over the world. It is based on natural fermentation when lactic acid bacteria (LAB) ferment water soluble carbohydrates to organic acids, mainly lactic acid, under anaerobic conditions. As a result, the pH decreases, inhibiting detrimental anaerobes, and so the moist forage is preserved (Weinberg *et al*., 2004; Chen & Weinberg, 2009). In order to achieve this goal, various silage additives have been used to improve the fermentation process and silage quality. Adding lactic acid bacteria (LAB) to ensiled forages ensures the presence of enough lactic acid bacteria to cause a rapid reduction in pH, which may contribute to eliminating undesirable fermentation and reduce proteolysis (Kung *et al*., 2003). A combination of anaerobic condition and acidity protects the forage from the proliferation of deleterious bacteria and fungi, and it also increases the palatability of the forage due to lactic acid production (Weinberg *et al*., 2003; Filya, 2003). Ando *et al.* (2006) also reported that the addition of lactic acid bacteria increased the digestibility of dry matter, organic matter, and crude protein and also voluntary intake of silage.

In terms of storage, silage is more durable because of spoilage bacteria do not resistant to low pH so its availability and quality of feed can be assured. Silage can also be used as a probiotics and organic acids sources for livestocks as an alternative to antibiotics. In order to improve the ensiling process and to obtain a high-quality fermented product, various chemical and biological additives have been developed and used during silage fermentation. The biological additives are advantageous because they are safe and easy to use, non-corrosive to machinery, do not pollute the environment and are regarded as natural products (Filya *et al*., 2000). Sugar is a limiting factor in producing good-quality fermented products. Sugar mainly serves as a carbon source for microorganisms. Water-soluble carbohydrates (WSC) are main source of food for microorganism during silage fermentation, thus regarded as essential substrates for growth of LAB for proper fermentation and low levels may restrict LAB growth. The WSC contents of various materials, including vegetable waste, are usually low for adequate fermentation to produce good quality silage. Therefore, it is necessary to use some additives to increase the supply of available carbohydrate substrates for the growth of LAB or to inhibit the activity of aerobic bacteria and decrease the loss of WSC in the early stage of ensilage (Shao *et al*., 2003).

A successful ensiling process requires a minimum concentration of fermentable sugars (3%-5% in DM). However, the majority of carbohydrates in plants is in the form of fibrous polymers that make up the cell wall and are not fermented by lactic acid bacteria (LAB). In order to obtain the necessary level of fermentable water-soluble carbohydrates (WSC) for the lactic fermentation in crops which are low in WSC, the use of carbohydrate sources has been suggested.

Vegetable waste are more difficult to ensiled compared with other forages, such as grass, because its high moisture and low water soluble carbohydrates concentration. Low protein content makes vegetable waste need some additive before ensiled to increase its nutritive value as ruminant feed.

Research on the ensiling of vegetable waste is still limited, and the effects of different additives may vary from one to another, resulting variety of resulting silages. Therefore, the objective of this research was to evaluate the quality of vegetable waste silages, using rice bran, *onggok* (cassava flour waste) and pollard as carbohydrate sources.

## MATERIALS AND METHODS

Vegetable waste was collected from local traditional market in Bogor, consisted of corn husk, chinese cabbage and cabbage. After chopped into ±5 cm length, vegetable waste was air dried until water content decreased to 70%. It was sprayed with *Lactobacillus plantarum* 1A-2 as an innoculum and divided into equal portions for the application of treatments. All innocula were diluted with distilled water so that they were applied at the same rate (10 ml of solution/kg of vegetable waste).

Experiment was held in a randomized block design consisted of six treatments with 3 replications. Randomized block design was used because limited amount of vegetable waste available in one day. Replication was made in three different days, and used as block. Treatments were (T1) vegetable waste (67%) + rice bran (33%), (T2) vegetable waste (59%) + rice straw (8%) + rice bran (33%), (T3) vegetable waste (67%) + *onggok* (33%), (T4) vegetable waste (59%) + rice straw (8%) + *onggok* (33%), (T5) vegetable waste (67%) + pollard (33%), (T6) vegetable waste (59%) + rice straw (8%)+ pollard (33%). Vegetable waste was a mixture of corn husk (50%), chinese cabbage (25%) and cabbage (25%). Rice straw was chopped before used into ±6 cm length.

Each treatment was packed into ±15L plastic drum as silos in triplicate and sealed. Each silo contained around 14 kg of chopped vegetable waste. Silos were stored at room temperature. After 45 d ensiled, each silo were sampled for chemical and microbial analyses. The dry matter content was determined by oven drying for 48 h at 60 $^{o}$C. After drying, samples were ground through a 1-mm screen miller, and stored in glass bottle at room temperature for chemical (proximate and fiber) analysis. The quality of silages was evaluated by measuring pH, temperature, population of lactic acid bacteria and total acid production. Percentation of lactic acid was determine as total acid titrated measured by titration method (AOAC, 1995), because innoculum used in this experiment was homofermentative lactic acid bacteria. Nutrient characteristic was determined by proximate and fiber analysis at Laboratory of Feed Technology, IPB. Dry matter (DM), crude protein (CP), ether extract (EE), and organic matter (OM) were analyzed according to methods from AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the methods described by Van Soest *et al*. (1991).

Another portion of original sample was diluted with autoclaved distilled water and blended in high-speed blender for 30 seconds. The pH of the water extract was immediately determined using a pH meter. The diluted samples were enumerated for LAB on pour-plates using MRS agar (DeMan, Rogosa, and Sharpe) with TPC (Total Plate Count) method, cultivated at 30 °C for 48 h. Colonies were counted from plates of appropriate dilutions containing a minimum of 30 colonies. pH was immediately measured from the remainder of diluted sample using pH meter.

## RESULTS AND DISCUSSION

The dry matter content of a crop at ensiling has a strong influence on the rate of fermentation and resulting silages. Filya *et al.* (2004) and Sucu & Filya (2006) concluded that ensiling low dry matter forages will lead to higher fermentation losses. Vegetable waste generally is very high in water content and low in dry matter, ranged between 12%-14% (Hersom, 2006; Ozkul *et al*., 2011). The addition of rice straw in this experiment was to increased dry matter at ensiling, while addition of rice bran, *onggok* or pollard was to increased WSC content.

Quality of silage can be observed from the physical characteristics of the resulting silage. After 45 d of ensiling, vegetable waste silage in this study exhibiting a yellowish green color, fresh aroma and not slimy. There was only a few fungal contamination visually observed on the surface of the silage due to aerobic conditions. Those physical characteristic indicated a successful fermentation process. The most common fungal contamination found in silage are from genera *Fusarium*, *Penicillium*, *Aspergillus*, *Altenaria*, *Mucor*, and *Rhizopus* (Adam, 2008). Use of rice bran, onggok, and pollard as a source of carbohydrates in each treatment resulted only a little color differences of silages. Silage using onggok had a lighter color while silage using pollard and rice bran had a darker color. Physical appearance of vegetable waste silages from each treatment can be seen in Figure 1.

Other parameters for evaluating silage quality are the chemical (temperature, pH and total acid production) and microbiological (LAB population) characteristics of silage presented in Table 1. Temperature of silage at the end of fermentation was varies between treatments. The highest temperature was recorded (29 $^{o}$C) on silage with rice bran addition (T2), which significantly higher compared to others. Rise in silage temperature comes from the heat generated from metabolic activities.

One of the objectives of use of silage inoculants during ensiling is to promote lactic acid production, which subsequently reduces the pH to preserve the forage. The pH of all silages was below 4 suggesting that silages were well fermented. According to Haustein (2003), optimum pH value for silage is <4.2, whereas lactic acid bacteria still survive to preserve forages. Value of pH, total acid production, and population LAB were not significantly affected by different carbohydrate sources addition. Vegetable waste silages treated with *onggok* gave the lowest pH value (3.42 and 3.55), while the highest pH value was obtained from silages treated with rice bran (3.91 and 3.94). This condition may relate to the acidity of *onggok* which lower than rice bran and pollard.

Supplementation of additive for ensiling is common way for improving silage quality. Addition of WSC

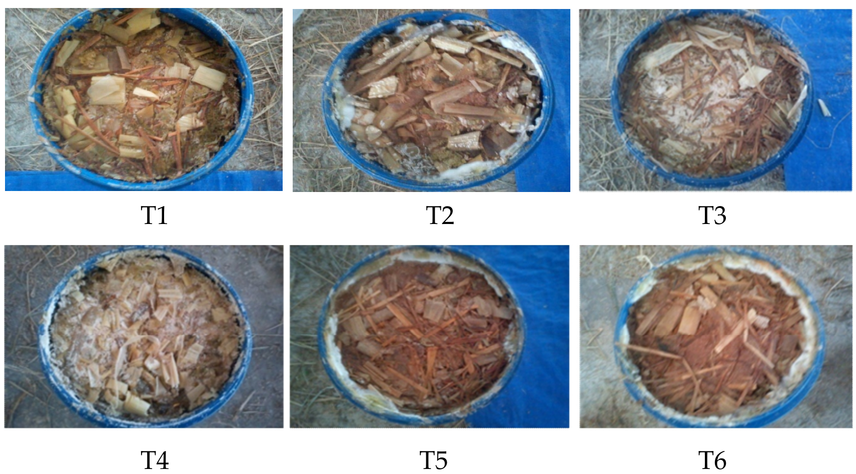


Figure 1. Physical appearance of vegetable waste silages. T1= vegetable waste (67%) + rice bran(33%); T2= vegetable waste (59%) + rice straw (8%) + rice bran (33%); T3= vegetable waste (67%) + onggok (33%); T4= vegetable waste (59%) + rice straw (8%) + onggok(33%); T5= vegetable waste (67%) + pollard (33%); T6= vegetable waste (59%) + rice straw (8%) + pollard (33%).

Table 1. Chemical and microbiological characteristics of vegetable waste silages

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Temperature ($^o$C) | 28.33±0.01$^b$ | 29.00±0.00$^b$ | 27.00±0.00$^a$ | 27.33±0.57$^a$ | 27.33±0.57$^a$ | 27.00±0.00$^a$ |
| pH | 3.91±0.01 | 3.94±0.04 | 3.42±0.46 | 3.55±0.42 | 3.91±0.09 | 3.72±0.19 |
| Lactic acid (%) | 0.41±0.10 | 0.30±0.05 | 0.23±0.01 | 0.29±0.11 | 0.30±0.06 | 0.36±0.11 |
| Lactic acid bacteria (cfu/g) | 7.10 x $10^8$ ±4.98 x$10^8$ | 10.40 x $10^9$ ±4.10 x $10^8$ | 6.97 x $10^8$ ±3.59 x $10^8$ | 16.5 x $10^9$ ±8.97 x $10^8$ | 6.71 x $10^8$ ±2.08 x $10^8$ | 5.48 x $10^8$ ±3.06 x $10^8$ |

Note: T1= vegetable waste (67%) + rice bran(33%); T2= vegetable waste (59%) + rice straw (8%) + rice bran (33%); T3= vegetable waste (67%) + onggok (33%); T4= vegetable waste (59%) + rice straw (8%) + onggok(33%); T5= vegetable waste (67%) + pollard (33%); T6= vegetable waste (59%) + rice straw (8%) + pollard (33%).

sources for ensiling resulted in producing sufficient lactic acid for rapid pH reduction and improving the fermentative quality of silage (Bureenok *et al.*, 2005), thus lactic acid production was correlated with pH value. Low WSC content means low nutrient sources served for LAB to produces lactic acid. Higher WSC content will produced silage with higher lactic acid concentration also reported by Yang *et al.* (2006) and Downing *et al.* (2008). Higher lactic acid production would result in lower pH value, but in this experiment, some silage with low lactic acid production also had low pH value. This result also reported by Addah *et al*. (2011), Contreras-Govea *et al*. (2011) and Vendramini *et al*. (2010) where higher lactic acid content in silages does not followed by lower pH. Previous studies have also indicated that lactate accumulation and its final concentration is the most reliable indicator of an ability of inoculants to improve the fermentation of silage (Hristov & McAllister, 2002). Lactic acid production in this study was ranged between 0.23%–0.41%, with no significant differences among treatments. Silages treated with *onggok*(T3) had lowest lactic acid production compared to other carbohydrate sources, this correlated with low WSC content of *onggok*. According to Despal *et al.* (2011) *onggok* have low WSC content (3.1%), while rice bran and pollard have higher WSC content (5.4% and 12.5%). The addition of WSC sources improve fermentable carbohydrate content of silages that provided a suitable environment for lactic acid bacteria to produce lactic acid and decreased final pH of silages (Nisa *et al.*, 2008; Saricicek & Kilic, 2011).

Inoculating vegetable waste with lactic acid bacteria prior to ensiling was aimed to produced a better quality silage, enhanced the desirable microflora and also decreased the growth of spoilage fungi or yeast (Amado *et al*., 2012). Inoculants were originally used to reduce pH and to avoid, or decrease, the risk of a clostridial fermentation by the native bacterial population (Wilkinson *et al*., 2003). *L. plantarum* 1A-2 added as inoculants during silage making to increased population of lactic acid bacteria, in order to stimulate lactic acid fermentation, accelerate the decrease in pH, and thus improve silage preservation of homofermentative lactic acid bacteria strains. They produce large amounts of lactic acid in the silage in a short time and stabilize it with minimal losses (Filya, 2003). Studies about addition of lactic acid bacteria for vegetable waste silages are limited, but several studies showed the beneficial effects of lactic acid bacteria for ensiling forages. Ratnakomala *et al.* (2006) reported that addition *L. plantarum* 1A-2 as inoculants for elephant grass silage resulted in lower pH and higher lactic acid than uninoculated silage. Similar findings have also been reported by Li *et al.* (2010) using *L. plantarum* for rice straw silage, Filya *et al.* (2007) for alfalfa silage, and Ando *et al.* (2006) for Guinea grass silage. Lactic acid produced by lactic acid bacteria will dominate fermentation and decreased pH, therefore can restrict the activity of spoilage bacteria like *Clostridia* (Bureenok *et al*., 2006). Combination of *L. plantarum* and carbohydrates source will result in better fermentation quality of legumes silages, compare with control silages without any addition (Heinritz *et al.*, 2012). In general, different carbohydrate sources had not influenced population of lactic acid bacteria significantly. The highest population of LAB was observed in silages with *onggok* addition (T4) (16.5 x $10^9$ cfu/g), while pollard addition (T6) produced silages with lowest population of LAB (5.48 x 10 cfu/g $^8$).

Nutrient characteristics of vegetable waste silages after 45 d of ensiling are shown in Table 2. Use of pollard as carbohydrate source gave the highest CP content (14.52%) than other treatments, while the use of *onggok* provided the lowest CP content of vegetable waste silages (3.56%). This result is correlated with CP content of carbohydrate sources added in silage production. Pollard has CP content around 15.53%, while rice bran and onggok have only CP content around 11.21% and 2.02% (Despal *et al*., 2011). Sapienza & Bolsen (1993) stated that CP content of good quality silage ranged between 10.50%-15.20%. Above the optimum range will result in poor silage quality and cannot be stored for a long time due to biochemical reactions between amino acids and sugars causing the Maillard reaction which produced brown silage. CP content in this experiment is still low, therefore increase level of high protein source addition is needed in the future.

The contents of CF and EE in vegetable waste treated with rice bran were the highest in all silages (28.15% and 1.70) as predicted, because rice bran had the highest CF and EE content compared with other carbohydrate

Table 2. Nutrient composition of vegetable waste silages

| Nutrient (%) | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Dry matter (DM) | 86.57 | 88.44 | 88.54 | 90.75 | 91.64 | 87.21 |
| Ash | 26.46 | 15.37 | 2.52 | 7.59 | 5.84 | 9.61 |
| Crude protein (CP) | 7.51 | 9.40 | 3.56 | 4.59 | 14.52 | 13.45 |
| Crude fiber (CF) | 25.79 | 28.15 | 14.26 | 19.76 | 10.99 | 16.41 |
| Ether extract (EE) | 1.70 | 0.30 | 0.12 | 1.01 | 0.19 | 1.15 |
| Non fiber extract (NFE) | 25.11 | 35.22 | 68.08 | 57.80 | 60.10 | 46.59 |

Note: T1= vegetable waste (67%) + rice bran(33%); T2= vegetable waste (59%) + rice straw (8%) + rice bran (33%); T3= vegetable waste (67%) + onggok (33%); T4= vegetable waste (59%) + rice straw (8%) + onggok(33%); T5= vegetable waste (67%) + pollard (33%); T6= vegetable waste (59%) + rice straw (8%) + pollard (33%).

Table 3. Fiber composition of vegetable waste silages

| Fiber (%) | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Neutral detergent fiber (NDF) | 58.77 | 72.69 | 58.28 | 76.44 | 52.96 | 56.33 |
| Acid detergent fiber (ADF) | 51.59 | 49.65 | 33.37 | 72.26 | 19.87 | 32.43 |
| Hemicellulose | 7.18 | 23.04 | 24.91 | 4.18 | 33.09 | 23.90 |
| Cellulose | 33.44 | 30.41 | 23.71 | 49.55 | 16.70 | 22.38 |
| Lignin | 10.77 | 10.24 | 9.03 | 18.14 | 2.67 | 5.27 |

Note: T1= vegetable waste (67%) + rice bran(33%); T2= vegetable waste (59%) + rice straw (8%) + rice bran (33%); T3= vegetable waste (67%) + onggok (33%); T4= vegetable waste (59%) + rice straw (8%) + onggok(33%); T5= vegetable waste (67%) + pollard (33%); T6= vegetable waste (59%) + rice straw (8%) + pollard (33%).

sources used in this experiment. Silage with EE content more than 2% will be easily contaminated and classified as bad quality of silage (Sapienza & Bolsen, 2003). The highest EE in this experiment was 1.70%, indicating a good quality of silage.

Fiber compositions of silages were not affected significantly by treatments (Table 3). Fiber carbohydrates, mainly hemicelluloses and cellulose, was used as substrates for microorganisms during ensiling (McDonald *et al.,* 1991). The use of rice straw as silage material increased the value of NDF, compared with silages without rice straw (T2 vs T1, T4 vs T3, T7 vs T6), and also increased lignin content of silages except for T2. Neutral detergent fiber (NDF) is related to the filling effects of feeds in the rumen. Pollard addition (T5 and T6) resulted in the lowest NDF, ADF, cellulose and lignin contents in silage, but exhibiting the highest level of hemicelluloses (33.09%). Use of *onggok* as carbohydrate source resulting in the highest cellulose content (49.55%) and lowest hemicelluloses (4.18%).

## CONCLUSION

All carbohydrate sources (rice bran, *onggok* (cassava byproduct), and pollard) used in this experiment can be used as silage ingredient resulting in good vegetable waste silage.

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